Susceptibility of adult mosquitoes to insecticides in aqueous sucrose baits

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ABSTRACT: Mosquitoes characteristically feed on plant-derived carbohydrates and honeydew just after emergence and intermittently during their lives. Development of toxic baits focusing on this carbohydrate-seeking behavior may potentially contribute to localized control. In the present study, ten insecticides were fed to female *Culex quinquefasciatus, Anopheles quadrimaculatus*, and *Aedes taeniorhynchus* in a 10% sucrose solution. Active ingredients representative of five classes of insecticides (pyrethroids, phenylpyroles, pyrroles, neonicotinoids, and macrocyclic lactones) were selected for comparison with commercial formulations used to facilitate incorporation of active ingredients into aqueous sucrose solutions. Sucrose as a phagostimulant significantly enhanced mortality to toxicants. In general, the most effective active ingredients were fipronil, deltamethrin and imidacloprid, followed by spinosad, thiamethoxam, bifenthrin, permethrin, and cyfluthrin. The least effective ingredients were chlorfenapyr and ivermectin. For some of the ingredients tested, *Cx. quinquefasciatus* was the least susceptible species. One-day-old male *Cx. quinquefasciatus* were more susceptible than females; however, no differences existed between one- and seven-day-old mosquitoes. There were no differences in susceptibility between unfed and gravid ten-day-old female *Cx. quinquefasciatus* to bifenthrin. In conclusion, several pesticides from different classes of compounds have potential for use in development of toxic baits for mosquitoes. ***Journal of Vector Ecology* 36 (1): 59-67. 2011.**

Keyword Index: Mosquito, insecticide, sugar bait, *Culex, Aedes, Anopheles.*

INTRODUCTION

Plant-derived sugars and honeydew provide important components of mosquito nutrition and contribute to energy for survival, flight duration, maintaining nutritional reserves and may enhance fecundity (Nayar and Sauerman 1971, Nayar and Sauerman 1975a, 1975b, Foster 1995, Breigel 2003). Obtaining a sugar meal is critical for male mosquitoes of all species and occurs frequently, possibly several times a day (Gary and Foster 2006). Female mosquitoes typically sugar-feed just after emergence with a stronger dominance of attraction to sugar sources over host responses, and then intermittently as needed (Foster 1995, Reisen et al. 1986, Foster and Takken 2004, Gary and Foster 2006). Nectar is considered a more readily available source of energy than blood and mosquitoes sugar feed more frequently than blood feed (Nayar and Van Handel 1971). For some anthropophilic species such as *Aedes aegypti* L. and *Anopheles gambiae* Giles, sugar feeding may play a lesser role in female nutrition compared to human blood (Foster 1995, Breigel 2003).

This predisposition of mosquitoes to seek and return to carbohydrate sources through their life presents an opportunity for use of an attractant/toxicant bait for localized population reduction. Lea (1965) first reported on the utilization of mosquito sugar-feeding behavior of mosquitoes as a control method when he observed enhanced mortality of *Ae. aegypti* after feeding upon a malathion-sugar solution. Incorporation of *Bacillus sphaericus* Meyer and Neide spores with the sucrose/dye solutions provided further evidence with *Culex pipiens* L. (Schlein and Pener 1990) and phlebotomine sand flies (Robert et al. 1997) that this approach could be used to cause mortality as well as provide a pathway for dissemination of mosquito-borne pathogens. Feral mosquitoes feeding on a dried sucrose solution mixed with dye and spinosad applied to flowers on trees at desert oases has resulted in reduction of populations compared to control locations (Müller and Schlein 2006). Using this same approach but including fruit juice as an attractant and spraying the solution on vegetation around a larval habitat provided control of local *Cx. pipiens* (Müller et al. 2010). Similar applications of a sucrose solution combined with boric acid and applied to vegetation resulted in reduced populations and landing rates of *Aedes albopictus* (Skuse) and *Culex nigripalpus* (Xue et al. 2006). Lastly, use of aqueous sugar solutions in bait stations containing boric acid, fipronil (Xue et al. 2008), or spinosad (Muller and Schlein 2008) resulted in significant reductions of local mosquito populations.

While numerous insecticides have been evaluated for their toxicity to mosquitoes and other biting flies by means of external contact, relatively little is known about the oral toxicity of different active ingredients. Previous studies have reported efficacy of ingested solutions of malathion (Lea 1965), boric acid (Xue and Barnard 2003), spinosad (Müller and Schlein 2006), and fipronil (Xue et al. 2008), however, comparative studies of active ingredients or target pest species are few. As part of an overall strategy of providing alternative methods for mosquito control, we evaluated the efficacy of active ingredients for potential use in toxic aqueous baits for adult mosquitoes.

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14. ABSTRACT

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MATERIALS AND METHODS

Mosquitoes

Culex quinquefasciatus Say, *Aedes taeniorhynchus* (Wiedemann) and *Anopheles quadrimaculatus* (Say) were reared in the laboratory following the methods of Gerberg et al. (1994). Adults were maintained in screen cages with a 10% sucrose solution provided continuously. Cages were held at 27-29° C and 70-85% RH under a photoperiod of 14:10 (L:D).

Assays

Assays were conducted using disposable plastic cups (100 ml) covered by a piece of fabric screening fastened by an elastic band. Mosquitoes were immobilized on a chill table (4° C), sexed, and counted with ten mosquitoes placed in each cup. Mosquitoes were allowed to recover for at least one h before the test. Baits were presented as a 1 cm length of cotton dental wick (Unipack Medical Corp., Commerce, CA) saturated with 1 ml of solution and placed on top of the screen lid of each cup. Cups were held at 70-85% RH and 25-27° C during the tests. Mortality was difficult to determine with mosquitoes that were moribund so knockdown (inability to stand) (KD) was determined as an index of mortality. Mosquitoes that were knocked down were not observed to revive.

Enhanced knockdown with sucrose

To determine if mortality was enhanced with the addition of sucrose in an aqueous solution of pesticide, knockdown of five to ten-day-old female *Cx. quinquefasciatus* to bifenthrin (Table 1) in solutions of sucrose was compared to that in solutions of water. Solutions of bifenthrin were prepared using 10% sucrose (w/v) or water over the range of 0.1-1000 mg/liter of active ingredient (AI). Knockdown was evaluated at four and 24 h. Bifenthrin was selected as a representative insecticide for this assay because of the lack of phagorepellency at high doses.

Effect of sex and physiological condition

To determine if sex and physiological condition affected response, bifenthrin-sucrose solutions were first used to compare susceptibility of teneral (one-day-old) *Cx. quinquefasciatus* males and females not exposed to sucrose. Additionally, comparisons were made between seven-day-old males and females (exposed to sucrose) and between ten-day-old gravid (five days post blood-feeding), and non-blood-fed females. Mosquitoes were exposed to a range of concentrations and sucrose controls as described above and KD_{50} and KD_{90} values estimated.

Evaluation of insecticide efficacy

Toxicants were selected to represent a range of active ingredients belonging to several classes of insecticides (Table 1). As many pesticides have low solubility in water, commercial formulations were used to facilitate the incorporation of the AI into the aqueous sugar solution. Stock solutions (1000 mg/liter AI) and subsequent dilutions of insecticides were made in 10% sucrose/water (w:v) solutions with 10% sucrose as the negative control (following Pridgeon et al. 2008). At least five concentrations of AI were tested of each insecticide and each test was replicated at least five times. Observations of knockdown were made at one, four, and 24 h after initial exposure. Females of *Cx. quinquefasciatus, Ae. taeniorhynchus,* and *An. quadrimaculatus* that were seven to 14-days-old with constant access to sucrose solution were used for bioassays.

Analysis

Data for comparisons between bifenthrin solutions made with sucrose or water were arcsine transformed and compared by paired *t*-test. Data for comparisons between active ingredients were analyzed by probit regression analysis (PoloPlus, LeOra Software 2003) after correction for control responses (if needed) using Abbot's formula (Abbott 1925). Data were presented at KD_{50} and KD_{90} values with (95% confidence levels) for different materials or life stages. Values were considered significantly different ($P < 0.05$) if confidence intervals did not overlap.

RESULTS

Mortality of *Cx. quinquefasciatus* females was significantly greater when exposed to bifenthrin-sucrose concentrations of >1 mg/liter compared with bifenthrin-water mixtures at four h (Figure 1). At 24 h, similar trends were observed, but there was no difference in bifenthrin mortality between sucrose or water at 1000 mg/liter. With many of the toxic doses, hyper-extended abdomens were observed (Figure 2). These mosquitoes did not recover and were dead at 24 h.

Age and sex of *Cx. quinquefasciatus* did affect susceptibility to bifenthrin in sucrose solutions (Table 2). Male mosquitoes were more susceptible than females at one day of age (KD_{50}) but not at 7 days. Unfed and gravid ten-day-old mosquitoes were equally susceptible (Table 2). There was no difference in the KD_{50} values between one-, seven- and ten-day-old females. One-day-old males were more susceptible than seven-day-old males based on KD_{50} values.

Because mortality at one and four h was generally low, KD_{50} and KD_{90} values were difficult to estimate with any accuracy, therefore only 24 h data are presented. Based on KD_{50} values, *Cx. quinquefasciatus* were most susceptible to fipronil > imidacloprid > deltamethrin, spinosad, thiamethoxam, and permethrin > bifenthrin > cyfluthrin > chlorfenapyr and ivermectin (Table 3). Females of *An. quadrimaculatus* were most susceptible to imidacloprid, fipronil, and deltamethrin > thiamethoxam, cyfluthrin, bifenthrin, spinosad, and permethrin > chlorfenapyr and ivermectin (Table 4). Females of *Ae. taeniorhynchus* were most susceptible to imidacloprid, permethrin, thiamethoxam, deltamethrin, and fipronil > bifenthrin and spinosad > cyfluthrin > chlorfenapyr and ivermectin (Table 5). These rankings were generally similar when KD_{90} values were used, except that some repellency was observed

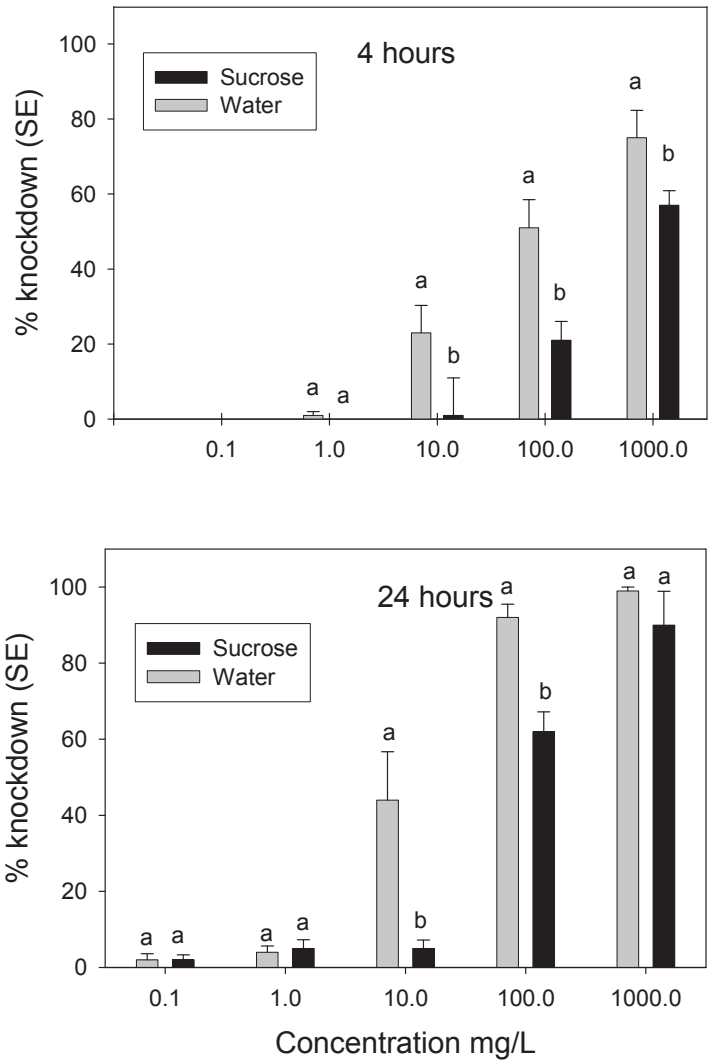

Figure 1. Knockdown of *Cx. quinquefasciatus* females exposed to bifenthrin combined with 10% sucrose or water (control). At each concentration, bars with similar letters were not significantly different (*t*-test, $P < 0.05$).

Figure 2. Characteristic hyper-extended abdomens of mosquitoes fed on toxic sugar baits.

Table 1. Active ingredients and commercial formulations used for toxic baits.

Class	Active ingredient	Formulation	% AI	Source
Pyrethroid	Bifenthrin	Talstar®	7.9%	FMC Corporation, Philadelphia PA
	Cyfluthrin	Tempo®	11.8%	Bayer Corporation, Kansas City MO
	Deltamethrin	Suspend®SC	4.75%	Bayer Environmental Health Sciences, Research Triangle Park NC
	Permethrin	Dragnet®	36.8%	FMC Corporation, Philadelphia PA
Phenylpyrazole	Fipronil	Termidor®SC	9.1%	BASF, Research Triangle Park NC
Pyrrole	Chlorfenapyr	Phantom®	21.45%	BASF, Research Triangle Park NC
Neonicotinoid	Imidacloprid	QuickBayt™	0.5%	Bayer HealthCare, Shawnee Mission KS
	Thiamethoxam	Platinum®	21.6%	Syngenta Crop Protection Inc. Greensboro NC
Macrocyclic lactone	Spinosad	Elecktor®	2.46%	Elanco Animal Health, Indianapolis IN
	Ivermectin	Ivomec®	0.1%	Merial Limited, Duluth GA

Table 2. Effect of age and stage of *Culex quinquefasciatus* on efficacy of bifenthrin in 10% sucrose solutions.

Age	Stage	KD_{50} (CL) [a]	Slope (SE)	N
1 day	Female	2.1 (1.8-2.6)a	2.66 (0.16)	900
	Male	1.5 (1.4-1.6)b	5.90 (0.56)	800
7 day	Female	3.0 (2.0-4.4)a	1.56 (0.08)	700
	Male	4.2 (2.0-7.4)a	1.77 (0.14)	900
10 day	Unfed female	1.9 (1.4-2.4)a	3.70 (0.37)	600
	Gravid female	1.8 (1.4-2.2)a	1.52 (0.12)	700

Means within each column and age followed by different letters are significantly different.
[a]Values are presented as mg of active ingredient per liter of sucrose solution.

at higher doses with some compounds and species resulting in high variation.

In general, of the pyrethroids, deltamethrin appeared to be the most toxic with cyfluthrin the least toxic. Fipronil, deltamethrin, and imidacloprid were the most toxic compounds across the three species. The neonicotinoid compounds thiamethoxam and imidacloprid were generally equivalent, although the former was more toxic in the case of *Ae. taeniorhynchus*. For all species, the least toxic compound was ivermectin, which was 49-, 3- and 387- fold less toxic than permethrin for *Cx. quinquefasciatus*, *An. quadrimaculatus*, and *Ae. taeniorhynchus*, respectively. The second least toxic compound was chlorfenapyr.

For the three species tested, there was no difference in susceptibility to spinosad or fipronil. However, *Cx. quinquefasciatus* was less susceptible than both *Ae. taeniorhynchus* and *An. quadrimaculatus* to bifenthrin, cyfluthrin, chlorfenapyr, thiamethoxam, and ivermectin.

DISCUSSION

This study provides a list of potential pesticides for use in a toxic bait approach for mosquitoes and other biting flies. With concerns of resistance in field populations, availability of effective toxicants with different modes of action may expand the potential for development of a toxic bait system. Other concerns with the selection of such a delivery system include the environmental impact of the toxicant, effects on non-target organisms, and stability under delivery conditions in the field. Development of a successful toxic bait system involves several components including visual and olfactory lures to attract mosquitoes to the bait. Moreover, mechanical exclusion of larger non-target insects, phagostimulants to maximize uptake of toxicant, a pesticide effective against target species formulated to optimize delivery of the active ingredient, minimal environmental impact, and avoidance of UV degradation will need to be addressed. The Environmental Protection Agency has designated some active ingredients

Table 3. Toxicity of active ingredients presented in sucrose solution for *Culex quinquefasciatus* females after 24-h exposure.

Active ingredient	N	KD_{50} (CL)[a]	KD_{90} (CL)[a]	Slope (SE)
Pyrethroid				
Bifenthrin	550	12.0 (9.1-15.5)	54.6 (33.6-88.3)	1.94 (0.15)
Cyfluthrin	750	23.9 (18.5-31.5)	124.5 (84.0-218.3)	1.79 (0.11)
Deltamethrin	500	1.5 (0.7-2.5)	77.8 (43.8-168.9)	1.57 (0.15)
Permethrin	550	7.8 (2.2-12.9)	36.5 (24.4-74.4)	1.91 (0.32)
Phenylpyrazole				
Fipronil	500	0.1 (0.1-0.2)	2.7 (1.2-11.4)	0.90 (0.11)
Pyrrole				
Chlorfenapyr	500	204.6 (146.4-300.4)	1103.1 (653.0-2588.3)	1.75 (0.15)
Neonicotinoid				
Imidacloprid	500	0.8 (0.3-1.9)	494.2 (150.9-3472.2)	0.46 (0.05)
Thiamethoxam	550	5.1 (2.2-7.9)	316.01 (120.9-1636.9)	0.71 (0.09)
Macrocyclic lactone				
Spinosad	790	1.7 (1.1-2.7)	15.3 (8.4-38.8)	1.37 (0.18)
Ivermectin	500	382.9 (291.7-587.9)	1254.4 (755.4-3621.5)	2.48 (0.35)

[a]Values are presented as mg of active ingredient per liter of sucrose solution.

as "reduced risk" compounds on the basis of factors such as hazards to humans and other animals, environmental fate, and photostability. In the current study, the compounds that fit into that category were fipronil, imidacloprid, thiamethoxam, and spinosad. Insecticide baits for mosquitoes using a phagostimulant such as sucrose can be effective in eliciting mortality. Based on toxicity, fipronil, imidacloprid, thiamethoxam, and spinosad appear to have potential for such an approach.

Pyrethroids are commonly registered for mosquito control with permethrin being the most widely used and available in the most formulations. Pyrethroids are sodium channel modulators and although highly toxic to mosquitoes, concerns of resistance may mitigate their potential use in toxic baits. Of the insecticides evaluated in our study, all pyrethroids were highly toxic with deltamethrin and permethrin the most toxic, bifenthrin moderately toxic, and cyfluthrin the least toxic. In a comparison of 19 active ingredients applied topically, Pridgeon et al. (2008) reported that permethrin was the second most toxic compound against adults of the three species. *Culex quinquefasciatus* was the least susceptible species to permethrin with *Ae. aegypti* the most susceptible species. Liu et al. (2004) reported that deltamethrin was more toxic than permethrin against adults of a susceptible strain of *Cx. quinquefasciatus*. Cyfluthrin and permethrin were equally toxic against a susceptible strain of *Cx. quinquefasciatus* (Nanzi et al. 2005).

Age and sex of mosquito did affect susceptibility to bifenthrin, with one-day-old males more susceptible than one-day-old females and more susceptible than seven-day-old males. This difference may be related to the larger size of females compared to male *Cx. quinquefasciatus* upon emergence and the lower glycogen reserves of teneral males compared with older males[1]. Differences in susceptibility to insecticides also existed between mosquito species. Similar to Pridgeon et al. (2008), we observed differences between species with some compounds less toxic to *Cx. quinquefasciatus* than to *An. quadrimaculatus* and *Ae. taeniorhynchus*. This observation was also supported by Corbel et al. (2004) who reported that dinotefuran was less toxic to *Cx. quinquefasciatus* than to *Ae. aeygpti* and *An. gambiae*. Our results demonstrate that the general tolerance of *Cx. quinquefasciatus* to pesticides compared to other species previously noted for tarsal contact (Curtis et al. 1996) and to dorsal application (Pridgeon et al. 2008), can

[1]Vrzal, E.M. 2009. The effects of various carbohydrate sources on longevity and nutritional reserves of *Culex quinquefasicatus* Say, *Culex nigripalpus* Theobald and *Culex salinarius* Coquillett. M.S. thesis. University of Florida, Gainesville. 93 pp.

Table 4. Toxicity of active ingredients presented in sucrose solution for *Anopheles quadrimaculatus* females after 24-h exposure.

Active ingredient	N	KD_{50} (CL) [a]	KD_{90} (CL) [a]	Slope (SE)
Pyrethroid				
Bifenthrin	500	2.8 (1.5-4.4)	9.2 (5.5-29.4)	2.47 (0.24)
Cyfluthrin	550	2.0 (0.7-5.6)	81.5 (24.1-621.7)	0.80 (0.05)
Deltamethrin	550	0.2 (0.1-0.6)	49.1 (10.1-103.5)	0.54 (0.05)
Permethrin	500	4.6 (3.1-9.3)	25.7 (11.7-160.9)	1.71 (0.23)
Phenylpyrazole				
Fipronil	500	0.2 (0.1-0.3)	0.7 (0.5-1.8)	2.35 (0.28)
Pyrrole				
Chlorfenapyr	500	24.4 (19.2-31.2)	90.2 (65.5-141.8)	2.26 (0.19)
Neonicotinoid				
Imidacloprid	500	0.03 (0.001-0.1)	2.1 (0.6-14.3)	0.70 (0.07)
Thiamethoxam	550	0.8 (0.6-0.9)	3.6 (2.9-4.8)	1.90 (0.23)
Macrocyclic lactone				
Spinosad	650	3.6 (1.7-7.6)	37.6 (15.6-172.6)	1.25 (0.09)
Ivermectin	500	14.2 (9.5-20.3)	141.6 (86.3-293.5)	1.28 (0.11)

[a]Values are presented as mg of active ingredient per liter of sucrose solution.

Table 5. Toxicity of active ingredients presented in sucrose solution for *Aedes taeniorhynchus* females after 24-h exposure.

Active ingredient	N	KD_{50} (CL) [a]	KD_{90} (CL) [a]	Slope (SE)
Pyrethroid				
Bifenthrin	550	2.1 (1.1-3.8)	13.5 (7.1-47.6)	1.60 (0.13)
Cyfluthrin	550	12.7 (10.4-15.6)	56.7 (42.2-83.8)	1.97 (0.16)
Deltamethrin	700	0.2 (0.1-1.3)	110.8 (17.4-443.9)	0.49 (0.03)
Permethrin	500	0.1 (0.1-0.2)	0.8 (0.5-1.9)	1.71 (0.13)
Phenylpyrazole				
Fipronil	400	0.3 (0.2 -0.5)	0.6 (0.4-1.2)	4.98 (0.52)
Pyrrole				
Chlorfenapyr	500	25.2 (21.5-29.4)	43.9 (36.5-58.9)	5.31 (0.62)
Neonicotinoid				
Imidacloprid	750	0.06 (0.001-0.2)	47.6 (12.5-523.5)	0.44 (0.04)
Thiamethoxam	550	0.1 (0.05-0.2)	0.9 (0.7-1.5)	1.44 (0.24)
Macrocyclic lactone				
Spinosad	650	4.1 (1.3-10.8)	32.2 (13.7-302.4)	1.25 (0.09)
Ivermectin	500	38.7 (29.3-51.1)	131.3 (92.2-224.1)	2.41 (0.20)

[a]Values are presented as mg of active ingredient per liter of sucrose solution.

be extended to oral toxicity. These results underscore the need for testing across species when evaluating the efficacy of toxicants for mosquitoes.

Neonicotinoid insecticides are agonists of the nicotinic acetylcholine receptor and have low toxicity to mammals, birds, and fish (Tomizawa and Casida 2005). Because they are generally stomach poisons, many have low contact toxicity and are most effective against piercing-sucking insects. Corbel et al. (2004) evaluated neonicotinoid dinotefuran as a topical against three species of mosquitoes and reported lower toxicity than deltamethrin but concluded that neonicotinods were potential candidates for disease vector control, particularly in areas where resistance to other insecticides is high. In our study, we evaluated two neonicotinoids, imidacloprid and thiamethoxam. The known tolerance of imidacloprid in some insect predators is advantageous with respect to non-target concerns (Bozsik 2006). Pridgeon et al. (2008) reported that imidacloprid applied topically was moderately toxic and lower in toxicity than permethrin for the three mosquito species tested. Paul et al. (2006) reported low mortality to imidicloprid treatments of *Ae. aegypti* in treated bottle assays. Liu et al. (2004) reported that susceptible strains were as susceptible to imidacloprid as to permethrin in larval assays. In our studies, imidacloprid was highly effective and as toxic as deltamethrin and permethrin for *Cx. quinquefasciatus*; it was also one of the most toxic compounds for *An. quadrimaculatus* and *Ae. taeniorhynchus*. Thiamethoxam was as toxic as permethrin for all species and one of the most toxic compounds for *An. quadrimaculatus*. Thiamethoxam combined with sugar has also been reported as highly toxic to house flies (Kristensen and Jespersen 2008) and eye gnats (Jiang and Mulla 2006). Thiamethoxam-treated spheres have been reported to be effective against blueberry maggot flies (Ayyappath et al. 2000). These neonicotinoid compounds are effective as both contact and stomach poisons and appear to be promising toxicants in an ingested bait system.

Macrocyclic lactones are products or chemical derivatives of soil micro-organisms and include the spinosyns and avermectins, both of which have contact and stomach effects. Spinosad is considered a naturally-derived biorational insecticide with low toxic effects for mammals, avians, predatory beneficial insects, and for the environment in general (Liu et al. 1999, Williams et al. 2003, Galvan et al. 2006). Spinosad is used commercially with sucrose for control of various tephritid fruit flies (Prokopy et al. 2003, Yee and Chapman 2009). Lui et al. (2004) reported relatively low toxicity of a susceptible strain of *Cx. quinquefasciatus* to spinosad, but when used against field strains resistant to permethrin and other insecticides, spinosad was one of the most toxic compounds tested. These authors concluded that spinosad could be important for mosquito vector control, particularly for mitigation of development of resistance. Pridgeon et al. (2008) reported that spinosad was slightly less toxic than permethrin against all three species of mosquitoes tested, with *Cx. quinquefasciatus* being the least susceptible. Spinosad has also been reported as effective against mosquito larvae (Darriet and Corbel 2006, Jiang and Mulla 2009).

Previous studies have reported on effective delivery of spinosad in sucrose as oral baits. A combination of sucrose and spinosad evaluated by Jiang and Mulla (2006) was toxic to eye gnats but over 25-fold less toxic than thimethoxam and half as toxic as imidacloprid. Dry sugar baits containing spinosad have also been found to be eight times more toxic than imidacloprid baits against house flies (White et al. 2007). Romi et al. (2006) reported oral toxicity of spinosad in a 5% sucrose solution with 100% mortality at 24 h of female *Ae. aegypti* at 100 ppm and for *Anopheles stephensi* Liston and *Culex pipiens* L. at 1000 ppm. In field studies, dried as well as aqueous sucrose and spinosad baits have reduced local populations (Müller and Schlein 2006, 2008, Müller et al. 2010).

The second macrocyclic lactone in our study, ivermectin (chloride channel activator), at systemic doses in mammalian blood is clearly toxic to mosquitoes (Pampiglione et al. 1985, Jones et al. 1992, Bockarie et al. 1999, Chaccour et al. 2010) producing high mortality at 24 h for high doses. In our study, ivermectin as a component of a sugar bait was the least toxic of compounds tested.

Chlorfenapyr, as a pyrrole, is an uncoupler of oxidative phosphorylation and acts as a contact and stomach poison. In laboratory and field trials, it has been reported to be moderately efficacious against pyrethroid-resistant mosquitoes (N'Guessan et al. 2009, Oliver et al. 2010) and as such was recommended to be considered as a resistance management tool to circumvent or slow development of resistance. Paul et al. (2006), using tarsal contact assays, concluded that chlorfenapyr was moderately toxic to adult mosquitoes compared with permethrin. Our results with sucrose and chlorfenapyr mixtures indicated that chlorfenapyr was less toxic than many of the other compounds examined. Fipronil is a phenylpyrazole acting as a gamma amino butyric acid (GABA)-gated chloride channel agonist with both contact and stomach effects (Cole et al. 1993).

Fipronil was one of the most efficacious compounds in our study, 78-fold more toxic than permethrin for *Cx. quinquefasciatus*, and 23-fold for *An. quadrimaculatus,* but not different for *Ae. taeniorhynchus*. Liu et al. (2004) reported similar toxicity of fipronil and permethrin for *Cx. quinquefasciatus* larvae. Based on topical application of adults, higher efficacy of fipronil than permethrin was reported by Pridgeon et al. (2008) for *Ae. aegypti* (106-fold) and *Cx. quinquefasciatus* (ca. 4,800-fold), but not for *An. quadrimaculatus*. While direct comparisons between studies are difficult because of differences in methods of delivery and doses absorbed are different, the general trend remains that fipronil appears to be at least as toxic as permethrin. Use of fipronil in sugar bait stations reduced landing counts of *Ae. aegypti* and *Ae. taeniorhynchus* in screened cages but did not impact field populations of mosquitoes (Xue et al. 2008).

Sucrose enhanced uptake of solutions containing insecticide as seen with higher mortality from ingestion of

solutions containing sugar. Mortality was not considered to be a result of the lack of ingestion as a result of repellency due to high survival of water-deprived control mosquitoes at 24 hr and the abundance of abdomens bloated with sugar solution in all treatments. At high doses, mortality did not always reach 100%, possibly because of repellency. Jiang and Mulla (2006) also provide evidence of sucrose as an important phagostimulant for the ingestion of insecticide solutions.

These results indicate that there are several compounds from different classes of insecticides that are effective in eliciting high levels of mosquito mortality through ingestion of the insecticide as a sucrose bait. Formulations of insecticides used in this study were not developed for delivery as aqueous sucrose baits and therefore are not optimized for this delivery method. However, delivery of formulated compounds was uniform throughout the test and a relative comparison of compounds is presented. A primary factor determining efficacy of an insecticide is dose. Most studies use standardized methods (i.e., topical application) that ensure an exact dose is delivered to each individual being evaluated. In contrast, it is very difficult to determine the exact amount of insecticide reaching the target in bait studies. Although this uncertainty introduces more variation in the resulting data, it provides a realistic indication of the doses necessary to kill the target mosquitoes and the degree to which the mosquitoes will ingest a lethal dose.

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